

CAMPYLOBACTER AND HELICOBACTER IN THE ETIOLOGY OF GASTROINTESTINAL DISEASES

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Abstract – The order *Campylobacterales* comprises two genera: *Campylobacter* and *Helicobacter*, with a widespread distribution in both humans and animals. They are Gram-negative, spiral, helical and microaerophilic bacteria, with an optimal growth temperature of 37°C for *H. pylori* and 42°C for *C. jejuni* strains. While *Helicobacter pylori* are restricted to humans, other helicobacter species can be found in different mammals and occasionally in humans. Several *Campylobacter* species are recognized as human pathogens, while distinct species are pathogenic only occasionally, in children, the elderly and immunocompromised patients. Campylobacters and helicobacters are well adapted to the living conditions inside the gastrointestinal tract, where they can cause diseases as a consequence of inflammation. In addition, they are related to certain extraintestinal diseases, post-infectious sequels, malignancy and autoimmunity. Different clinical presentations of human disorders may be the consequences of the diversity in host immune response, bacterial genome, endotoxin activity as well as specific bacterial virulence factors.

Key words: *Campylobacter*, *Helicobacter*, gastrointestinal infection, human

INTRODUCTION

Originally discovered as etiological agents of animal diseases, campylobacters appear to be one of the most important human pathogens. Although they were initially named vibrios, campylobacters underwent taxonomic changes until their contemporary positioning. At one time, *Helicobacter* was placed in the *Campylobacter* genus as *Campylobacter pyloridis*, corr. *pylori*. Based on extensive genomic investigations (Lau et al., 1987; Paster et Dewhirst, 1988; Majewski et Goodwin, 1988), the two bacteria were finally separated, first as different genera and then as distinctive families. Genus *Helicobacter* G+C content is 35-44% while G+C content of genus *Campylobacter* is 29-38% (Godwin et al. 1989).

Campylobacter – a historical perspective

The end of the 19th century was an important period in the development of microbiology, including the identification of spiral gastric bacteria. In 1886, Theodor Escherich described a spiral bacteria in the colon of children who had died from a disease he termed 'cholera infantum'. However, in early days, campylobacter bacteria were not recognized as a cause of diarrhea and methods for its successful isolation from stool samples were not available for decades. Many years later Elisabeth O'King who understood their importance, insisted on the development of specific cultivation techniques. "Unfortunately, such a method was not developed in her lifetime, but her vision and diligence paved the way" (Butzler, 2004).

In the 40 years after their recognition, campylobacters were referred only to veterinary medicine. In 1947, Vinzent for the first time published data on vibrio isolation from the blood of 3 pregnant women with fever of unknown etiology, which in two cases ended with abortions (Vinzent et al., 1946). Before that report, in May in 1938, Illinois, USA, the first well-documented human campylobacter infection occurred. It was a diarrhea outbreak caused by milk consumption. Fecal culture was negative in all patients while in 31 patients the presence of bacteria resembling '*V. jejuni*' was detected microscopically. In 13 patients, this microorganism was recovered from the blood stream (Levy, 1946).

All species that belong to the genus *Campylobacter*, as well as related taxa, in 1991 were classified in the same phylogenetic group, rRNA superfamily VI. At that time, the genus *Campylobacter* was comprised of 15 species (Vandamme et al., 1991). Contemporary taxonomy described these microorganisms as members of the following categories: Kingdom: Bacteria, Phylum: *Proteobacteria*, Class: *Epsilonproteobacteria*, Order: *Campylobacteriales*, Family *Campylobacteraceae*, Genera: *Campylobacter* and *Arcobacter*. Nevertheless, constant scientific efforts are revealing novel campylobacter species and candidates: *C. avium* (poultry) *C. canadensis* (*Grus americana*), *C. cuniculorum* (rabbits), *C. hominis* (humans), *C. insulinea* (sea mammalian), *C. lanienae* (workers at abattoirs), *C. peloridis*, *Campylobacter lari*-like strains (shellfish and humans), *Campylobacter troglodytis* (human) (Euzéby, 2011).

Clinical presentations of Campylobacter infections

At least 12 species of campylobacters can cause disease in humans, but major credit belongs to the thermophilic organisms: *Campylobacter jejuni*, *Campylobacter coli*, *Campylobacter lari* and *Campylobacter upsaliensis*. These are etiological agents of diarrhea in 4-15% of all age groups. Diarrhea can be bloody, with cramps and abdominal pain. In immunocompromised patients, *Campylobacter* can enter the blood stream and cause life-threatening infections. *Campylobacter* are more frequently isolated in children and

young adults than in other age groups. Although most infections are mild and self-limiting, resolving within a few days without antibiotic treatment, severe or prolonged infections can occur in the young, elderly and in individuals with compromised immunity (Blaser, 1997; Blaser et Reller, 1981).

The consequences of infection can be extraintestinal manifestations such as transient bacteremia, localized infections including septic arthritis, meningitis (Blaser, 1997), peritonitis (Van den Enden et al., 1990), cholecystitis (Drion et al., 1988), hepatitis (Braun et al., 2008), pancreatitis (De Bois et al., 1989), abscesses, fulminate sepsis (Blaser et Reller, 1981, Fernández-Cruz et al., 2010). Several cases of myocarditis as a complication of *C. jejuni* infection have been reported (Braun et al., 2008, Kratzer et al., 2010). As an effect of *Campylobacter enteritis*, hemolytic uremic syndrome can occur (Bruce et al., 1983). Long-term repercussions of campylobacter infection are Guillain-Barré syndrome (GBS) (Miljković-Selimović et al., 2010), or a variant of GBS, Miller Fisher syndrome (MFS) (Willison et Yuki, 2002), musculoskeletal disorders (Hannu et al., 2002), inflammatory bowel disease (IBD) (Garcia Rodriguez et al., 2006, Kalischuk et al., 2010] and immunoproliferative small intestinal disease (IPSID) (Lecuit et al., 2004).

In campylobacter intestinal infections, significant roles are assumed by a variety of adhesion factors (*Campylobacter* adhesion to fibronectin (CadF), fibronectin-like protein A (FlpA), *Campylobacter* adhesion protein A (CapA), Jejuni lipoprotein A (Jlpa), periplasmic protein, Pei, Ellison, Blaser (PEB), Cj496c, major outer membrane protein, (MOMP), capsular polysaccharide (CPS), lipooligosaccharide, (LOS)), invasiveness (*Campylobacter* invasive proteins (Cia) proteins), toxicity (cytolethal distending toxin - CDT), genetic variability (Nielsen, 2010), biofilm formation (Reuter et al., 2010) and quorum sensing (Elvers et Park, 2002). *C. jejuni* is the first and almost unique prokaryote that contains both O- and N-linked glycosylation systems. Studies investigating the biological role of N-linked glycosylation connect this biochemical pathway to bacterial virulence,

while O-linked glycosylation has been essential for successful flagellin assembly and motility, adhesion, invasion and virulence *in vivo* (Guerry et al., 2006).

Based on clinical syndromes, two mechanisms by which *Campylobacter* can induce disease were postulated: adherence of *Campylobacter* to the intestine and toxin production that alters the fluid resorption capacity of the intestine, resulting in secretory diarrhea or bacterial invasion and replication within the intestinal mucosa accompanied by an inflammatory response resulting in blood-containing, inflammatory diarrhea (Janssen et al., 2008).

C. jejuni bacteria initially colonize the small bowel and then move to the colon which is the target organ. These bacteria can adhere to epithelial cells via a number of different adhesions, but the relative significance of each to disease remains uncertain. JlpA is a surface exposed lipoprotein that has been shown to bind to the surface-exposed heat shock protein Hsp90a on Hep-2 epithelial cells resulting in the activation of NF- κ B and p38 mitogen-activated protein kinase (MAPK). CadF mediates adhesion by binding to the cell matrix protein, fibronectin. This protein has recently been shown to be critical in the activation of Rac1 and Cdc43 in INT407 cells. Another reported adhesion is PEB1, a periplasmic ABC-binding protein that binds aspartate and glutamate and that is required for the intestinal colonization of mice. More recently, a putative autotransporter, CapA, has been described that also plays a role in the adherence and invasion of Caco-2 cells *in vitro* (Poly et Guerry, 2008).

The flagella filament of *Campylobacter* appears to function as a type III secretion organelle that secretes a number of proteins. The Cia proteins are secreted through the flagella filament upon contact with a eukaryotic cell or a signal from the eukaryotic cell. Invasiveness is promoted by the bacterial ability to disrupt the tight junctions of epithelial cells. The ability to break the epithelial cell barrier either via transcellular (through epithelial cell invasion) or paracellular (via tight junctions) routes allows the bacterium to move to the basolateral surface and either reinvade

the epithelial cell or be taken up into macrophages. *C. jejuni* can replicate intracellularly in macrophages and induce apoptosis (Poly and Guerry, 2008).

It has been demonstrated that *C. jejuni* infection activates intestinal epithelial NF- κ B (Zilbauer et al., 2005) and that the surface protein JlpA promotes bacterial adhesion leading to NF- κ B and p38MAP kinase activation. The crucial mediators of interleukin (IL)-8 production are *C. jejuni* adhesion/invasion and the presence of CDT. IL-8 induction can be provided by an ERK pathway, while ERK and p38 MAP kinases are involved in *C. jejuni*-mediated host responses (Watson et al., 2005). It is possible that human-pathogen interaction can be fulfilled by pathogen associated molecular patterns (PAMPs), which interact with toll-like receptors (TLRs), a form of host pattern recognition receptor (PRRs) (Sander-son et al., 2007). Nucleotide oligomerization domain 1 (NOD1), a cytoplasmic, intracellular PRR, is highlighted as a major PRR involved in *C. jejuni*-mediated epithelial responses (Zilbauer et al., 2007). An important protective role during *C. jejuni* infection is played by the antimicrobial peptides β -defensins (Zilbauer et al., 2005). These molecules damage the structure of *C. jejuni*, potentially contributing to the enhanced bacterial clearing and self-limiting nature of disease in immunocompetent patients (Zilbauer et al., 2005; Zilbauer et al., 2009).

Although *Campylobacter* can circumvent the activation of innate immunity via TLR5 and TLR9, innate immune mechanisms are essential for host defense, since mice defective in downstream TLR signaling and NF κ B-gene-deleted mice display an enhanced susceptibility to *Campylobacter* infection (Janssen et al., 2008)

In the etiology of neurological postinfectious sequels (such as GBS), the molecular mimicry between *C. jejuni* LOS and human gangliosides, described by Yuki and co-workers (Yuki et al, 1993), is of paramount importance in the genesis of cross-reactive antibodies and initialization of autoimmune response. In addition, it has been shown that specific types of the LOS biosynthesis gene locus

involved in sialylation are important for the ganglioside mimicry and the induction of antiganglioside antibodies (Godschalk et al., 2004; Godschalk et al., 2007). Preceding *C. jejuni* infection is often caused by serotype O:19 (Miljković-Selimović et al., 2010). Other serotypes also associated with GBS are: O:41, O:1, O:2, O:4, O:4 complex (4, 13, 16, 43, 50), O:5, O:10, O:16, O:23, O:37, O:44, O:64 O:35 and O:13/65 (51). Miller Fisher syndrome (MFS), which is characterized by ophthalmoplegia, ataxia and areflexia, can be related to *C. jejuni* O:10 and O:2. Conditions related to MFS are Bickerstaff's brainstem encephalitis (BBE); acute ophthalmoparesis; ataxic GBS; and pharyngeal-cervical-brachial (PCB) weakness (Willison et Yuki, 2002). Some authors consider PCB, GBS, MFS and BBE to be forms of a continuous spectrum (Nagashima et al., 2007). Thus, besides neurological examination, specific microbiological and immunological tests are necessary in the etiological diagnosis of *C. jejuni* neurological postinfectious sequels. In addition, clinical, epidemiological and microbiological investigations are warranted in *C. jejuni* characterization and HS serotypes prevalence investigation in GBS etiology as well as in etiology of other postinfectious sequels.

C. jejuni is also associated with post-infectious musculoskeletal manifestations: reactive arthritis (ReA), sacroiliitis, enteropathic spondylitis, and undifferentiated spondylitis. Symptoms of reactive arthritis usually occur around 14 days after infection (range, 3 days to 6 weeks). Some investigations revealed that the percentage of patients with confirmed *C. jejuni* infection who develop ReA range from 0.7%, (1.8%, 2.0%, 2.6%, 7%,) to 30% (Ristić, 2010). A population study in southern Finland found that 7% of the participants developed ReA and 1% developed reactive tendinitis, enthesopathy or bursitis (Hannu et al., 2002). Investigation of patients with ReA showed that 24% (Mäiki-Ikola et al., 1991) or 63% (Soderlin et al., 2002) had antecedent *Campylobacter* infection. Several events participate in the immunopathogenesis of musculoskeletal disorders: a long-term production of IgA directed towards bacterial agents; diminished reactivity of peripheral T

lymphocytes to bacterial antigens from the digestive tract; chronic stimulation in the enteric lymphatic tissue provoked by bacterial antigens; survival of bacteria and its penetration into circulation enabled by weakened T cell defense (Toivanen and Toivanen, 2001). The association and role of HLA B27 are still vague, but several experimental models have been proposed for the pathogenesis of ReA (Marker-Hermann, 1998).

C. jejuni has been isolated from patients with IBD such as Crohn's disease (CD) and in patients with irritable bowel syndrome. *C. jejuni* enteric infection results in damage of the mucosal layer and disturbance in normal bacterial gut flora, which could lead to illness. It is possible that bacterial toxicity plays a leading role (Janssen et al., 2008). As IBD patients exhibit inflammatory responses to their commensal intestinal microflora, translocation of commensal bacteria across the intestinal epithelium may contribute to IBD pathogenesis. *C. jejuni*, regardless of its own invasiveness, promotes the translocation of noninvasive bacteria across the intestinal epithelium via a lipid raft-mediated transcellular process (Kalischuk et al., 2009). In addition, *C. jejuni* may utilize M cells for the transcytosis promotion of non-invasive bacteria (Kalischuk et al., 2010). *C. jejuni* infection concomitant with intestinal inflammation and exposure to interferon gamma (IFN)- γ would result in an intensive bacterial translocation across the intestinal epithelial monolayer, rapid loss of epithelial barrier integrity, which may be a key event in the pathogenesis of *C. jejuni*-mediated colitis and the development of bloody diarrhea (Rees et al., 2008). Not only *C. jejuni*, but also *C. concisus*, *C. showae*, *C. hominis*, *C. ureolyticus*, *C. hyointestinalis*, *C. rectus*, and *C. gracilis*, are described risk factors for the development of IBD through unknown mechanisms in adult patients with CD (Mahendran et al., 2011). In children with CD, the presence of *Campylobacter* species other than *C. jejuni* has also been detected: there was a significantly greater presence of *C. concisus* and antibodies to *C. concisus* in patients than in the control (Zhang et al., 2009). Nevertheless, a causal link between the bacterium and the pathogenic mechanisms of inflammation awaits further clarification.

An association between *C. jejuni* infection and irritable bowel syndrome was also observed. These enteric infections result in mucosa damage and disruption of the native gut flora, which could lead to prolonged bowel dysfunction. Increased enteroendocrine cells, T lymphocytes and gut permeability are acute changes following *Campylobacter* enteritis that can persist for more than a year and may contribute to post-diarrheal irritable bowel syndrome (PB-IBS) (Spiller et al., 2000). In addition, the long-term symptoms that occur in *Campylobacter* infection are significantly associated with bacterial toxicity on the cell culture (Thornley et al., 2001).

Since *C. jejuni* association with IPSID has been confirmed, it seems that *C. jejuni* can be considered as a possible candidate responsible for immunoproliferative states (Lecuit et al., 2004). The role of *C. jejuni* in IPSID may be similar to that played by *H. pylori* in gastric MALT lymphoma. The lymphoma cells synthesize defective α heavy chains as a response to tissue autoantigens. The uncontrolled autoantigenic stimulus results in a continuous differentiation of lymphoma cells into atypical plasma cells, while the removal of the bacterial T-cell stimulus inhibits the proliferation of lymphomatous B cells (Parsonnet et al., 2004).

Antimicrobial therapeutic intervention is warranted in severe forms of disease, extraintestinal manifestations and in post-infective sequels (Blaser et Engberg, 2008). However, antibiotic intervention in food-producing animals in therapy, disease prevention and as growth promoters, has generally introduced problems of resistance in thermophilic campylobacter species. Resistance is an increasing problem, especially against fluoroquinolones, tetracyclines and macrolides, the drugs applied in diarrhea therapy. In one investigation of Serbian strains, gentamicin and chloramphenicol sensitivity was 100%, while resistance to erythromycin, tetracycline, ciprofloxacin and nalidixic acid occurred in 2.4%, 9.9%, 29.8% and 33.3% of strains, respectively. Resistance to tetracycline and especially ciprofloxacin emphasizes the need for continual sensitivity testing. (Miljković-Selimović et al., 2009).

Detection and strain characterization

Although biochemical tests are commonly applied in the identification of thermophilic campylobacters, their identification power is restricted to specific biochemical pathway expression in particular strains. Thus, PCR and PCR-based methods are considered to be a valid and reproducible approach for the detection of *hipO*, *glyA*, *asp*, *ceuE*, *cad*, *fur*, *cdtABC*, *ceuB-E*, *fliY* and other genes (Volokhov et al., 2003; Kabir et al., 2011; Persson and Olsen, 2005).

Phenotypic characterization of strains can be performed by biotyping and serotyping. The biotyping of *C. jejuni* and *C. coli* includes hippurate hydrolysis, H₂S and DNase production, while serotyping is based on heat labile (HL serotyping) and heat stabile antigens (HS serotyping). Investigation of the HS and HL serotypes of *C. jejuni* and *C. coli* isolated in Serbia, confirmed their clonal diversity, with no predominant ones (Miljković-Selimović et al., 2010).

Current typing methods are usually based on molecular techniques: pulse field gel electrophoresis (PFGE), amplified fragment length polymorphism (AFLP), flagellin gene restriction fragment length polymorphism analysis (*flaA*-RFLP), multi locus sequence typing (MLST), multiple-locus variable number tandem repeat analysis (MLVA), and DNA Microarray with comparative genomic hybridization (CGH) (Wieland, 2006). The existence of a strong association of genotypes with particular hosts is noted, and is greater than the geographic signal. These findings are consistent with local and international transmission of host-associated lineages of *Campylobacter* among food animal species (Sheppard et al., 2009).

To understand the molecular mechanisms involved in *C. jejuni* adaptation to its environment (oxidative stress, temperature, biofilms), innovative technologies, including proteomic, transcriptomic, lipidomic and genomic approaches, are needed (Seal et al., 2007). Still, little is known regarding the role of individual proteins in virulence: adhesion, colonization and toxicity, bacterial response to changes in the environment and human host and subcellu-

lar locations of most proteins. *C. jejuni*, unlike most other bacteria, is able to modify post-translationally proteins, the analysis of which represents a challenge in understanding this organism at the proteomic and cellular levels (Scott and Cordwell, 2009). Proteomics are also useful for characterizing phenotypic variation among *Campylobacter* spp. isolates. The different gene products potentially involved in the robust colonization of chickens by *Campylobacter* spp. appear to conform to recently identified expression patterns in biofilm or agar-adapted isolates (Seal et al., 2007).

Helicobacter discovery

The gastric spiral bacteria were first described in 1893 when Bizzozero, an Italian pathologist, discovered helical bacteria in the canine stomach. In 1896, Salomon reported similar findings in the stomachs of cats and mice. The presence of spiral bacteria in the human gastric system was detected in 1906, where, for the first time, Krienitz observed these organisms in patients with gastric carcinoma. Nine years later, Rosenow and Sanford found spiral microorganisms in the stomachs of patients with gastric and duodenal ulceration (Buckley and O'Moraint, 1998). The modern era in *H. pylori* investigation began with the Marshall and Warren *H. pylori* research (Marshall and Warren, 1984) in humans, and with their tenacity in changing the dogma of ulcer disease etiology which finally led to the revision of medical text books.

The genus *Helicobacter* was described in 1989 (Goodwin et al., 1989) and with *Wolinella* species belongs to the family *Helicobacteraceae*. Members of the genus can be classified in gastric and enterohepatic *Helicobacter* taxa. Based on 16S rRNA sequences, the phylogenetic tree is represented with 18 validated *Helicobacter* species, two candidate species, and nine additional provisional species (Solnic and Vandamme, 2001). *H. baculiformis* is the last formally named *Helicobacter* species isolated from the stomach of a cat (Baele et al., 2008). *H. callitrichis* and *H. macacae* are other isolates that have not been validated so far, but that are likely to represent a new helicobacters (Moyaert et al., 2008).

Helicobacter species characterized in the last decade are *H. anseris* (goose), *H. aurati* (golden Syrian hamster), *H. baculiformis* (feline stomach mucosa), *H. brantae* (geese), *H. canadensis* (humans), *H. cetorum* (whales, dolphins), *H. cynogastricus* (canine gastric mucosa), *H. equorum* (horse feces), *H. ganmani* (laboratory mice), *H. marmotae* (livers of woodchucks and intestines of cats), *H. mastomyrinus* (liver and intestine of rodents), and *H. suis* (pig), *H. typhlonius* (murine) (Euzéby, 2011).

It is assumed that *H. pylori* spread from East Africa over the same time scale (58,000 years ago) as modern humans. Studies have confirmed that *H. pylori* had colonized human gaster before modern man left his cradle, since genetic diversity in *H. pylori* decreases with geographic distance from East Africa. In addition, bacterium and man had similar evolution patterns, following human migration from East Africa to Asia, and the neighboring regions: Oceania, Europe and America (Vale et al., 2009). The map of *H. pylori* strain diversity appears to be similar to that of humans (Covacci et al., 1999, Linz et al., 2007).

Differences among the strains were followed at the genomic level for detection of their geographic distribution. The *H. pylori rpoB* gene (coding RNA polymerase β subunit) presents allelic diversity between Asian and non-Asian strains (Lee et al., 2004). Moreover, allelic diversity according to the geographic distribution was found for the *babA* and *babB* genes (coding outer membrane proteins) (Pride and Blaser, 2002; Pride et al., 2001). The transposable element ISHp60 is also represented non-randomly with higher frequency in Latin America and lower in East Asia (Kersulyte et al., 2002). The *hopQ* (*omp27*) alleles additionally show high genetic variability: type I alleles from Western and Asian *H. pylori* strains were similar and markedly different from type II *hopQ*, which were frequently identified in Western *H. pylori* strains, but rarely in East Asian strains (Cao et al., 2005). Furthermore, the geographic distribution of *H. pylori* methyltransferases gives evidence of human host population isolation and migration (Vale et al., 2009) along with candidate virulence factors, *vacA*, *cagA* and

iceA, that also cluster in strains of particular geographic regions (Li et al., 2002).

Helicobacter capability to persist in the human niche for thousands of years may be attributed to the predicted versatile horizontal and vertical transmission routes and diversity of plausible reservoirs and sources. Thus, *H. pylori* transmission pathways in developing rural and developed urban areas appear to be different. In developed areas, person-to-person transmission within families may be dominant, while in the developing areas the transmission is probably much more complex. In rural areas, the transmission by contaminated food, water, or via intensive contact between infants and non-parental caretakers may have a greater influence than within family transmission (Vale and Vitor, 2010).

Although, *H. pylori* isolation in humans was intense in 2003 and 2004, there were two outbreaks of increased mortality associated with gastric bleeding and weight-loss in a captive colony of the Australian marsupial, the stripe-faced dunnart (*Sminthopsis macroura*). Histological examination revealed the presence of gastritis, and PCR analysis confirmed the presence of *H. pylori* infection in the stomachs of these marsupials. It was confirmed that the strain was positive for the important pathogenesis factor, *cagA*. For the first time an apparent reverse zoonotic infection of animals with *H. pylori* was described. Indeed, *H. pylori* spontaneously vanished from the animal population (Every et al., 2011).

H. pylori in the etiology of gastrointestinal diseases

Although, *H. pylori* colonizes the human gaster in approximately half of the human population, it causes persistent inflammation with symptoms only in a subgroup of patients (Ernst and Gold, 2000). An even smaller proportion of infected persons develops severe diseases, such as peptic ulcers, gastric MALT lymphoma or gastric carcinoma. Numerous studies have demonstrated the phenotypic and genotypic diversity in *H. pylori* strains, which is responsible for different types of inflammatory re-

sponses in the host as well as versatile clinical outcomes (Kusters et al., 2006).

H. pylori in non-malignant gaster diseases

Non-malignant diseases associated with *H. pylori* infection are gastritis, peptic ulcer, gastroesophageal reflux disease, gastric polyps, nonsteroidal-anti-inflammatory drug/aspirin-induced gastric injury and functional dyspepsia (Furuta et Delchier, 2009).

The defined virulence factors of *H. pylori* are cytotoxin-associated gene pathogenicity island (*cagPAI*), vacuolating cytotoxin (*VacA*), adhesion factors and outer membrane proteins (blood group antigen binding adhesion (*BabA* or *HopS*)), outer inflammatory protein adhesion (*OipA*), sialic acid-binding adhesion (*SabA* or *HopP*) and lipopolysaccharides (LPS) (Kusters, 2006).

In the host, *H. pylori* induces both innate and specific immune responses, which in turn determines the infection outcomes. The humoral immune response does not have a protective role in *H. pylori* infection, while cell immunity predominates and protects from diseases. Innate epithelial defense depends on TLR and NOD-like receptor (NLR) activation which induces a *H. pylori* specific T helper (Th)1 immune response. Innate epithelial defense of gastric mucosa from *H. pylori* infection depends on TLR and NLR activation, which is responsible for a *H. pylori* specific Th1 immune response. Infiltration of the gastric mucosal layer with inflammatory cells is a frequent finding in *H. pylori* infection. The degree of mucosal damage is in correlation with neutrophile infiltration. It seems that the *H. pylori* neutrophile activating protein (HP-NAP) is the key factor in the generation of Th1 response and interleukin synthesis in monocytes, dendritic cells, and neutrophiles by TLR2 activation. Although bacterial LPS of Gram-negative pathogens recognizes TLR4 on the surface of epithelial cells, *H. pylori* LPS activates TLR2 rather than TLR4, thus activating NF- κ B (Del Giudice et al., 2001). *H. pylori* flagella cannot activate the TLR5 receptor (Andersen-Nissen et al., 2005), also *vacA* does not appear to be a significant player in the first step

of innate immune recognition mediated by TLR4 or TLR5 (Garza-González et al., 2008). In *H. pylori* infection and Th1 activation, the synthesized proinflammatory cytokines are IFN- γ , IL-12, IL-18 and tumor necrosis factor (TNF)- α . The degree of gastritis intensity is in correlation with TNF- α and IFN- γ expression. Peptic ulceration is associated with *H. pylori* specific local gastric Th1-cell responses (Bergman et al., 2006). A strong Th1 mucosal response is associated with the progression of gastric mucosa damage, occurrence of atrophic gastritis and gastric adenocarcinoma (Kusters et al., 2006).

On the contrary, in the subgroup of patients with asymptomatic chronic gastritis who fortunately account for the 80-90% of individuals infected but without apparent disease, most *H. pylori*-specific gastric T cells are Th0 cells, which secrete both Th1 and Th2 cytokines such as IFN- γ and IL-4 (D'Elis et al., 1997). Thus, data obtained from humans indicate that *H. pylori*-infected individuals who can overcome the initial Th1-cell-dominated response, mount a mixed Th1- and Th2-cell response to *H. pylori* in their gastric mucosa, which maintain persistent colonization without developing clinical disease (Bergman et al., 2006). This indicates that most infected people switch from an acute gastric *H. pylori*-specific response mediated by Th1 cells, to a response that is mediated by Th1 and Th2 cells. It seems that the *H. pylori* phase variants that bind C-type lectin that is a cell-surface receptor on dendritic cells (DC-SIGN), suppress the development of Th cells into Th1 cells through IL-10 (Bergman et al., 2004). This event might facilitate the switch and be selected by the host. DC-SIGN-binding variants of *H. pylori* are selectively bound by DC-SIGN-expressing DCs that protrude from the gastric epithelium, and these cells subsequently migrate to the gastric lymph nodes, where they suppress the development of Th cells into Th1 cells (Bergman et al., 2006).

Increased regulatory T cell (Treg) activation by bacterial antigens is one of the defense mechanisms of *H. pylori* that enable bacterial immune evasion from host immunity. Treg suppress antibody response and T-cell response either by cell contact or

by TGF (transforming growth factor)- β and IL-10. *H. pylori* gastritis is associated to Treg accumulation. In peptic ulcer disease, elevated Treg secreting IL-10 in gastric mucosa control the inflammation enabling bacterial persistence in gastric mucosa. Treg cells have a protective role for mucosa from extensive gastric inflammation on one hand, while on the other, they stimulate the gastric colonization and persistence of *H. pylori* infection (Kusters et al., 2006).

H. pylori in malignant gastric diseases

The role of *H. pylori* in human malignant diseases is at present undisputable. The International Agency for Research on Cancer has classified *H. pylori* as a class I carcinogen (IARC 1994). Nevertheless, tumor progression occurs only in a subset of individuals and depends on the host response as well as genetic variation of the bacteria. *H. pylori* virulence factors are key features in carcinogenesis. All *H. pylori* strains possess the *vacA* gene, but not all of them induce vacuolation. Two major polymorphic regions of the *vacA* gene are the signal region (s1 or s2) and the mid-region (m1 or m2). Malignancy is associated with s1/m1 strains and some s1/m2 strains. Rhead and associates identified a new *vacA* polymorphic site, designated as the intermediate (i) region, and two sequence types (i1 and i2). It was shown that s1/m1 and s1/m2 strains were exclusively i1 and s2/m2 exclusively i2. Only the i region was determined to be an independent marker of gastric carcinoma (GC). The typing of the i region may be sufficient for the identification of all pathogenic forms of *vacA* and may be very useful in cancer prevention (Rhead et al., 2007).

The host genetic factors that drive immune mechanisms have great significance in carcinogenesis. A high level of pro-inflammatory cytokine IL-1 expression (IL-1 β) and upregulation of *IL1RN* (which encodes the receptor antagonist of IL-1 β) consecutively decrease gastric acid secretion and increase the risk for atrophic gastritis. These events associated with predominant corpus *H. pylori* colonization and pangastritis, occur in individuals with a rise in the risk of carcinogenesis (El-Omar et al., 2000; Furuta

et al., 2002). In addition to IL-1 gene-cluster polymorphisms, in TNF- α a pro-inflammatory cytokine, genotype TNF-A 308A, is associated with augmented TNF- α production and the increased risk of gastric carcinogenesis associated with *H. pylori* infection (El-Omar et al., 2003).

Expression of the anti-inflammatory cytokine IL-10 is determined by certain IL-10 gene haplotypes: haplotype GCC is associated with an elevated expression of IL-10, and thereby with an enhanced anti-inflammatory response. Haplotype ATA decreases the level of IL-10 favoring the pro-inflammatory response (Kusters et al., 2006). An increasing number of pro-inflammatory genotypes seem to increase progressively the risk of gastric cancer: the presence of three to four specific pro-carcinogenic polymorphisms increases the risk of gastric cancer 27-fold compared to an absence of these haplotypes (El-Omar et al., 2003). Thus, the development of *H. pylori*-associated pathology is correlated with the ratio of Th1 and Th2 subsets, and the pro- and anti-inflammatory responses are influenced by the genotypes of the host (Bergman et al., 2006).

Changes in epithelial cell signaling induced by *H. pylori* contribute to the gastric carcinogenic process. A combination of CagA-dependent and CagA-independent signaling, which is dependent on the bacteria type IV secretion system (TFSS) mediated by Jun N-terminal kinase (JNK) activation through β 1-integrin and Src, was required to stimulate cancer cell motility (Snider et al., 2008). Although both pathways are necessary, neither is sufficient by itself to alter the phenotype. The Cag A-independent signaling that occurs through the TFSS indicates that TFSS may play a more important role in host cell physiology than just the delivery of CagA from the bacterium into the host cell cytoplasm (Ferreira et al., 2008). Apoptosis, the process of cell-destruction, may be under the influence of the genotype of the infecting bacteria. Although *H. pylori* cagPAI-negative strains can induce this process, the expression of cagPAI promoted apoptosis more rapidly and increased DNA fragmentation in gastric epithelial cells (Minohara et al., 2007). The major *H. pylori*-induced

apoptotic pathway in gastric cancer cells requires the activation of caspases-3 and -9 (Zhang et al., 2007). *H. pylori* also impairs the E-cadherin and β -catenin cell adhesion complex, leading to aberrant activation of β -catenin in an animal model, and thus is involved in precancerous intestinal metaplasia progression. It was noted that the gene that encodes for β -catenin can function as an oncogene (Wang et al., 2008), and the increase in β -catenin production was recorded in people with some forms of carcinoma (Saldanha et al., 2004). In addition, matrix metalloproteinases (MMPs) are a family of nine or more highly homologous zinc-dependent endopeptidases that collectively cleave most if not all of the constituents of the extracellular matrix (Birkedal-Hansen et al., 1993). They have the potential to disrupt gastric stroma and to promote bacterial invasion. In MMP secretion, CagA-dependent and CagA-independent mechanisms are involved. In addition to the dysregulation of β -catenin production by the CagA-dependent mechanism, it was shown that CagA, and in particular the EPIYA motif, was required for optimal extracellular signal-regulated kinase (ERKs) activation and MMP-1 secretion (Ferreira et al., 2008).

In addition to genetic alterations, epigenetic changes are also involved in cancer development and progression. *H. pylori* infection may be linked to CpG (cytosine-phosphate-guanine sites) hypermethylation, a mechanism that is particularly relevant in cancer since it can interfere with the expression and activity of tumor suppressor genes. Consequently, the rates of promoter methylation of p16, E-cadherin, and death-associated protein kinase are significantly higher in the noncancerous gastric mucosa of gastric cancer patients (Kaise et al., 2008).

In the characterization of host immune response to cagA⁺ *H. pylori* infection, the association with Th1-mediated cellular immunity in earlier stages of GC was observed with a shift to the Th2-mediated humoral immunity in the advanced stages. When stimulated by *H. pylori*, T cells from GC patients produced high amounts of IL-10, an inhibitory cytokine of Th2 immune response. This production was low in *H. pylori*-infected asymptomatic individuals. Thus, it

was proposed that the increased production of suppressive cytokine IL-10 in *H. pylori*-infected GC patients might lead to a decreased cytotoxic antitumor T-cell response in the stomach, which may contribute to tumor progression (Ferreira et al. 2008).

In gastric MALT lymphoma, CD4+T cells that encounter the *H. pylori* antigens displayed by antigen-presenting cells stimulate the proliferation of neoplastic B cells. These B cells synthesize IgM, IgA, or IgG autoantibodies and differentiate to varying degrees into mature plasma cells (Parsonnet et al., 2004). Although gastric mucosa does not normally contain lymphoid tissue, MALT appears in response to colonization with *H. pylori*. In some cases, a monoclonal population of B cells may arise from this tissue and slowly proliferate to form a MALT lymphoma. In particular, diagnosis is based on histological appearance during routine microscopy and on demonstration of clonality by immunohistochemistry or molecular techniques, such as PCR. A major predictor for MALT response appears to be a particular translocation that is associated with *API2-MALT1* fusion. Translocation t(11;18)(q21;q21) generates a functional API2 (apoptosis inhibitor 2)-MALT1 fusion transcript (Ferreira et al., 2008). The former is involved in regulation of apoptosis, while the latter resembles a caspase-like protein. Together, the fusion leads to suppression of apoptosis (Kusters et al., 2006).

Extragastric manifestations of H. pylori infection

Extragastric manifestations of *H. pylori* infection can involve cardiovascular diseases, metabolic disorders, lung, hematologic and hepatobiliary diseases, gynecologic diseases and neurologic diseases (Moyaert et al., 2008). With respect to metabolic disorders, *H. pylori* colonization is associated with reduced circulating leptin levels, independent of body mass index (BMI), while fundic ghrelin and leptin levels are directly related. Long-term eradication of *H. pylori* infection is associated with a significant increase in BMI, lean and fat mass, a decrease in circulating ghrelin levels and an increase in leptin levels.

Non-pylori Helicobacter species (NPHS) in gastrointestinal diseases

H. heilmannii and *H. felis* have been rarely found in human stomach infections (Okoli et al., 2009). The gastritis observed with *H. heilmannii* infection tends to be less severe than that due to *H. pylori*. *H. heilmannii* has been found in association with duodenal ulceration, gastric ulceration, gastric carcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma. A surprisingly high rate (3.4%) of MALT lymphomas in *H. heilmannii*-infected patients has been noted (O'Rourke et al., 2001).

Helicobacters cultured from human diarrheal samples include *H. cinaedi*, *H. canis*, *H. pullorum*, *H. fennelliae*, *H. canadensis*, *H. rappini* and other unclassified but related organisms. Despite all efforts to clarify their etiological role, it still remains vague. The data obtained by studying symptomatic and asymptomatic homosexual males and experimental evidence on macaques implicate *H. cinaedi* and *H. fennelliae* in the etiology of human intestinal disease. *H. pullorum* and *H. canadensis* have been cultured from immunocompetent and immunodeficient human patients presenting with acute or chronic diarrhea. *H. rappini* has been isolated in a gastroenteritis index case, his 16-year-old asymptomatic daughter and a pet puppy. In addition, *H. canis* has been isolated from a child suffering from gastroenteritis and in fecal samples from healthy and diarrheic dogs. *H. pullorum* and *H. canadensis* have been cultured from immunocompetent and immunodeficient human patients presenting with acute or chronic diarrhea (O'Rourke et al., 2001).

Intestinal *Helicobacter* species can enter the bloodstream, and it would therefore be expected that it could enter the liver. Despite detection of helicobacter 16s rDNA in liver tissues by PCR, there have been no published data of helicobacter cultivation or ultrastructural identification. The results of studies have often been conflicting and it seems that differences are related to geographic origin of the data.

Murine models have clearly shown that if the normal immune balances are altered then mucosa-associated *Helicobacter* species can induce pathology similar to human IBD. This is possibly due to their location in mucous, the microbial niche closest to the susceptible mucosa. Whether an analogous process occurs in humans is unclear. *Helicobacter* species have not consistently been isolated from IBD patients. The presence of non-*pylori Helicobacter* species in the human colon is unknown as this ecological niche has been poorly studied (O'Rourke et al., 2001).

NPHS in systemic disorders

There have been numerous reports of bacteremia associated with *Helicobacter* species with several reports of recurrent infections. *H. cinaedi* and *H. rap-pini* are the organisms most frequently cultured from blood. The majority of cases occurred in patients infected with HIV, and having other predisposing factors such as alcoholism, end stage renal failure, carcinoma, diabetes, and primary immunodeficiencies: X-linked agammaglobulinemia and hypogammaglobulinemia. *Helicobacter* have also been isolated in pediatric bacteremia and, surprisingly, in healthy immunocompetent adults. The secondary sites of infection reported with helicobacter bacteremia include abdominal abscess, cellulitis, septic arthritis, meningitis, and pneumonia (O'Rourke et al., 2001).

Helicobacter detection and typing methods

There are numerous tests for the detection of *H. pylori* infection. At present, priority is given to the development and improvement of non-invasive techniques. Nevertheless, invasive tests are first applied for *H. pylori* detection. The gold standard in *H. pylori* diagnosis is gastric intubation, endoscopy and biopsy of gastric mucosa samples. *H. pylori* molecular identification methods can be applied for detection of virulence factors (Wen et al., 2007) and identification of macrolide resistance (Megraud, 2007).

Contemporary *H. pylori* typing methods are MLST, polymerase chain reaction – restriction frag-

ment length polymorphism (PCR-RFLP), AFLP, arbitrarily primed (AP)-PCR, repetitive extragenic palindromic DNA sequence-based PCR (REP-PCR), type specific PCR (*vacA*, *iceA*, *BabA*, 23S alleles), PCR-line probe assay *LiPA* (PCR-LiPA), restriction endonuclease analysis (REA), PFGE, ribotyping, CagA detection, IS605/606 detection, and plasmid profile analysis (Owen et al., 2001). Genomic methylation typing is useful to type bacteria that have a high number of expressed type II methyltransferases (MTase), such as *H. pylori*. Due to the type II restriction-and-modification (R-M) systems after acquisition, and due to the diversity of R-M systems in *H. pylori*, MTase from R-M systems was successfully used in the typing of *H. pylori* strains (Vale and Vitor, 2007).

A proteomic approach can be used in the identification of disease-specific bacterial biomarkers, targets for vaccines and serological diagnosis tests in *H. pylori* clinical isolates, throughout two dimensional electrophoresis (2DE) analyses, followed by mass spectrometry (MS) protein identification. The enhanced virulence of pediatric ulcerogenic strains results from a synergy between their natural ability to better adapt to the hostile human stomach and the expression of the established virulence factors (Vitoriano et al., 2011). Furthermore, studying the immunoproteome evidence has showed the variability of the antigenic pattern among *H. pylori* strains, which is the unusual extent of genetic heterogeneity that characterizes *H. pylori*, due to the unusually high rates of mutation and recombination events that occur *in vivo* (De Reuse et Bereswill, 2007).

CONCLUDING REMARKS

The order *Campylobacterales* is comprised of two families: *Helicobacteraceae* and *Campylobacteraceae*. These are Gram-negative, spiral, helical, microaerophilic organisms with optimal growth temperature at 37°C for *H. pylori*, and 42°C for *C. jejuni* strains. Both genera can be represented in mammals, including humans and birds as reservoirs. Diseases caused by *C. jejuni* are almost exclusively observed in humans, and *H. pylori* invades almost exclusively the human gaster. *H. pylori* and NPHS are involved in

the etiology of gastric disorders, *Campylobacter* and NPHS in the etiology of diarrhea, while *C. jejuni* and *H. pylori* play roles in the etiology of immunoproliferative diseases, inflammatory bowel disease and autoimmunity.

Different clinical presentations of human disorders may be the consequences of the differences in the host's immune responses and bacterial endotoxin activity (Moran, 2010). Since *H. pylori* endotoxin expressed underphosphorylation and underacylation of the lipid A, it has lower endotoxic activity and interaction with immune receptors is significantly lower, which may lead to the prolongation of *H. pylori* infection and chronicity. *C. jejuni* endotoxin is immunologically and endotoxically more active, thus contributing to diarrhea and acute inflammation (Moran, 2010). Moreover, flagellar proteins from both bacteria are defective in TLR5 activation.

However, both *H. pylori* and *C. jejuni* possess molecules that express cross-reactive epitopes with human tissue. *H. pylori* expresses mimicry of Lewis and some ABO blood group antigens involved in inflammation, gastric atrophy, and cancerogenesis. *C. jejuni* exhibits mimicry of gangliosides, crucial for the development of the GBS and MFS. It is possible that an innovative approach in strain characterization could be applied to describe both genera and would reveal more similarity than originally perceived or better explain the observed differences.

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